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Abstract: Obesity involves hypoxic adipose tissue and low-grade chronic inflammation. We investigated the impact of hypoxia on inflammatory response to TNF- in white and brown adipocytes. In response to TNF- , the expression of the inducible enzymes iNOS and COX-2 was prominently and selectively potentiated during hypoxia while only moderately under normoxia. Levels of their products, nitrite and prostaglandinE2 were elevated accordingly. NS398, a selective COX-2 inhibitor, reduced nitrite levels. The expression of PGC-1 , a transcriptional co-activator involved in mitochondrial biogenesis, and PPAR , a transcription factor involved in adipocyte homeostasis, was reduced by TNF- during hypoxia. These results suggest that hypoxia potentiates the inflammatory response by TNF- in both white and brown adipocytes and downregulates the transcription factors involved in adipocyte function.

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# Hypoxia potentiates tumor necrosis factor-α induced expression of inducible nitric oxide synthase and cyclooxygenase-2 in white and brown adipocytes

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**Running title: Inflammation in adipocytes during hypoxia**

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## Abstract

Obesity involves hypoxic adipose tissue and low-grade chronic inflammation. We investigated the impact of hypoxia on inflammatory response to TNF-α in white and brown adipocytes. In response to TNF-α, the expression of the inducible enzymes iNOS and COX-2 was prominently and selectively potentiated during hypoxia while only moderately under normoxia. Levels of their products, nitrite and prostaglandin$E_2$ were elevated accordingly. NS398, a selective COX-2 inhibitor, reduced nitrite levels. The expression of PGC-1α, a transcriptional co-activator involved in mitochondrial biogenesis, and PPARγ, a transcription factor involved in adipocyte homeostasis, was reduced by TNF-α during hypoxia. These results suggest that hypoxia potentiates the inflammatory response by TNF-α in both white and brown adipocytes and downregulates the transcription factors involved in adipocyte function.

**Keywords:** Adipose tissue, inducible nitric oxide synthase, cyclooxygenase-2, inflammation, hypoxia, mitochondrial dysfunction

## Introduction

Obesity, which is defined as excessive fat accumulation, is characterized by low-grade chronic inflammation of adipose tissue [1,2]. The obesity-driven inflammation in adipose tissue can progress to other metabolic tissues such as liver, pancreatic islets and muscles and mediates insulin-resistance [3].

In 1993, Hotamsigil et al., demonstrated that the pro-inflammatory cytokine, tumor necrosis factor-α (TNF-α) expression is elevated in adipose tissue of obese rodents [4]. Later, macrophages located in the adipose tissue were identified as the source of the TNF-α and also of inducible nitric oxide synthase (iNOS) [2]. TNF-α down-regulates the insulin-sensitive glucose transporter Glut4 in adipocytes [4], and indeed both pro-inflammatory proteins TNF-α and inducible nitric oxide synthase (iNOS) contribute to the development of obesity-induced insulin resistance in mice [5,6]. During normoxia stimulation with TNF-α in several cell lines led to a co-induction of iNOS and cyclooxygenase-2 (COX-2) [7,8]. Both iNOS and COX-2 are inducible enzymes that generate nitric oxide and prostaglandin, respectively and both act cooperatively and synergistically in pathological conditions [9]. In 1993 Salvemini and colleagues showed that a cross-talk exists between COX and NOS pathways and that NO activates COX enzymes to produce increased amounts of prostaglandins [10].

The presence of hypoxia in obese adipose tissue and its contribution to the initiation and progression of inflammation appears significant [11]. Hypoxia occurs when oxygen availability does not match the demand of the surrounding tissue, resulting in decreased oxygen tension [12]. In obese adipose tissue, the adipocytes become hypertrophied and the diameter of adipocytes increases up to 150 - 200 µm. Since the diffusion limit of oxygen is considered at 100 µm, hence pockets of hypoxia develop within the adipose tissue [11]. Moreover, in obesity vascularization in

adipose tissue is compromised which further restricts the oxygen availability to the adipocytes [13]. Hypoxia plays an underpinning role in triggering the infiltration of macrophages and secretion of pro-inflammatory cytokines such as tumour-necrosis factor (TNF), interleukin 6 (IL 6) and, CC-chemokine ligand 2 (CCL2; also known as MCP1) [14,15]. Alternatively, inflammation can also drive hypoxia and indeed inflammatory disease states are often characterized by tissue hypoxia or stabilization of hypoxic markers such as hypoxia-inducible factor (HIF) [16]. Thus, hypoxia and inflammation are intertwined at the molecular, cellular, and clinical levels [16] .

In the present study, using an in-vitro cell culture model of white and brown adipocytes, we characterized the effect of hypoxia on the inflammatory response. In particular, we investigated the molecules that are perturbed after treatment with TNF-α during hypoxia.

## Materials and methods

### Cell culture and differentiation

Murine 3T3-L1 preadipocytes (from ATCC, Manassas, VA, USA) at low passage were cultured in DMEM growth medium (Biochrom AG, Berlin, Germany) and differentiated to adipocytes as done previously [17]. Briefly, 3T3-L1 cells were grown to confluence in DMEM with 10% NCS. The cells were then given the differentiation cocktail comprising of 3-isobutyl-1 methylxanthine (0.5 mM), dexamethasone (0.25 µM), insulin (10 µg/ml) and rosiglitazone (2 µM) in DMEM with 10% FBS. After 3 days, the media was replaced with DMEM plus 10% FBS and insulin (1 µg/ml). After every $2^{nd}$ day, the media was changed and on $11^{th}$ day 80-90% of preadipocytes were differentiated. The differentiated 3T3-L1 adipocytes were then starved overnight in DMEM with FBS (0.5%) and adipocytes were then stimulated with TNF-α (10 ng/ml) in the presence of normoxia or hypoxia (1% $O_2$) for 24 h.

Immortalized preadipocytes (were provided by Prof. Dr. Johannes Klein) when differentiated resemble brown adipocytes [18] . The cells were grown in DMEM with 20% FBS. At 80% confluence, the cells were treated with induction media which comprised of T3 (1 nM), indomethacin (0.125 mM), dexamethasone (0.25 µM), 3-isobutyl-1 methylxanthine (0.5 mM), insulin (10 µg/ml) in DMEM with 20% FBS for 24 h. Cells were then changed to differentiation media which comprised of DMEM with 20% FBS, insulin (10 µg/ml) and T3 (1 nM). The differentiation media was changed after every 2 days. Differentiation was stopped on the $10^{th}$ day. Around 80-90% cells were differentiated to adipocytes and were starved overnight in DMEM with FBS (0.5%). The cells were then stimulated with TNF-α (10 ng/ml) in the presence of normoxia or hypoxia (1% $O_2$) for 24 h.

**RNA Isolation and Quantitative PCR**

RNA from cells was extracted using RNeasy® lipid tissue kit according to the protocol of the manufacturer (Qiagen, Hombrechtikon, Switzerland). RNA quantity was determined using Nanodrop 2000 Spectrophotometer. Reverse transcription of 50 ng RNA was perfomed using iScript™ Reverse Transcription Supermix according manufacture's recommendations (BioRad, Hercules, CA, USA). For quantitative RT-PCR, final cDNA concentration was adjusted to 10 ng in a 20 µl reaction volume. Each reaction was performed in duplicates using Bio-Rad CFX96 Real-Time System and iQ™ SYBR® Green Supermix (BioRad, Hercules, CA, USA) using specific primers. Gene was normalized to the reference gene *acidic ribosomal phosphoprotein* (*Arbp)* using the comparative C(T) method [19].

**Western Blotting**

Cells were homogenized using the lysis buffer containing Tris.Cl (50 mmol/L, pH 8.0), NaCl (150 mM), EDTA (0.5 mM), Triton X-100 (1%), protease inhibitor, and phosphatase inhibitor (Sigma, Buchs, Switzerland). Equal amounts of protein were loaded for SDS-PAGE, transferred to nitrocellulose and analyzed with the indicated antibodies. Immunoblotting was performed using the antibodies against iNOS, ERK2 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), COX-2 and COX-1 (Cayman Chemical Company, Ann Arbor, Michigan USA). The membranes were then incubated with the corresponding HRP-conjugated secondary antibodies (GE Healthcare, Buckinghamshire, UK). Labeled proteins were visualized on X-ray films using a chemiluminescence reaction.

**Immunoprecipitation**

3T3-L1 adipocytes were lysed with Tris.Cl (100 mmol/L, pH 7.4), NaCl (150 mM), glycerol (10%), Triton X-100 (1%), protease inhibitor, and phosphatase inhibitor (Sigma, Buchs, Switzerland). The co-immunoprecipitation was performed as mentioned before [20]. Briefly, 800 µl of lysis supernatant was pre-cleaned for non-specific binding by incubating with 50 µl protein A-Sepharose. After a quick spin, the supernatant was pipetted into a new eppendorf tube and incubated for overnight with COX-2 antibody at 4°C on a rotator. Subsequently, 80 µl of Protein A-Sepharose beads were added for 2 h under rotation at 4°C. The pellet was washed 3 times with lysis buffer and was boiled in SDS sample buffer and loaded to an 8% SDS PAGE. Immunoblotting was performed using iNOS antibody. The membranes were then incubated with the corresponding HRP-conjugated secondary antibodies (GE Healthcare, Buckinghamshire, UK). Labeled proteins were visualized on X-ray films using a chemiluminescence reaction.

**Measurement of nitrite**

Adipocytes kept in normoxia or hypoxia incubators were treated with or without TNF-α (10 ng/ml) for 24 h. The cells were pre-treated either with iNOS specific inhibitor 1400W (10 µM; Cayman chemical) or COX-2 specific inhibitor NS398 (10µM; Enzo Life Sciences, Lausen, Switzerland). The culture supernatants were collected to measure the released nitric oxide (NO). Levels of NO, in form of nitrite ($NO_2^-$) were determined using Griess reagent (1% sulfanilamide, 0.1% N-1-naphthylenediamine dihydrochloride, and 2.5% phosphoric acid) as done previously [21]. The absorbance was measured at 570 nm with a multi-mode microplate reader (Molecular Devices, Spectra Max M2, Bucher Biotec, Basel, Switzerland).

**Determination of $PGE_2$ production**

$PGE_2$ was measured in the culture media by competitive enzyme immunoassay according to the manufacturer's protocol (Cayman Chemical Co., Ann Arbor, MI, USA). Briefly, cells in normoxia or hypoxia were pre-treated either with 1400W (10 µM) or NS398 (10 µM) along with or without TNF-α (10 ng/ml) for 24 h. The cell culture medium was collected and centrifuged at 500 g for 5 min. The supernatant was used to measure the prostaglandin$E_2$ ($PGE_2$) levels.

**Statistical analysis**

Student's *t* test was used to compare data between two groups. Values are expressed as means ± SEM of three independent experiments. $P < 0.05$ was considered statistically significant.

## Results

### TNF-α increases the expression of iNos and Cox-2 in hypoxic adipocytes

Around 80% of 3T3-L1 preadipocytes were differentiated to adipocytes (Fig. 1A). In 3T3-L1 preadipocytes, stimulation with TNF-α increased the gene expression of iNos in normoxia and to a greater extent in hypoxia; however they were non-significant (Fig 1B). The gene expression of Cox-2 was minutely increased with TNF-α and the increase was similar between normoxic and hypoxic conditions (Fig. 1B). When differentiated 3T3-L1 adipocytes were used, stimulation with TNF-α increased the gene expression of iNos in normoxia and its expression was increased by 7-fold in hypoxia (Fig. 1C). The Cox-2 gene expression was also increased by 7-fold in adipocytes treated with TNF-α in the presence of hypoxia compared with normoxia (Fig. 1C). When brown adipocytes were used, treatment with TNF-α increased the expression of iNos gene and its expression was around 40-fold higher in hypoxia as compared with normoxia (Fig. 1D). The expression of Cox-2 gene in the presence of TNF-α and hypoxia was increased in brown adipocytes but was not significant compared with normoxia (Fig. 1D).

3T3-L1 adipocytes treated with TNF-α increased the protein expression of iNOS in normoxia. Adipocytes in hypoxia treated with TNF-α caused a robust increase in the protein expression of iNOS and COX-2 (Fig. 1E) but not of COX-1 (data not shown). A significant increase in expression of iNOS and COX-2 was also observed in brown adipocytes under hypoxia and TNF-α (Fig. 1F). Stimulation of 3T3-L1 preadipocytes with TNF-α in the presence of hypoxia did not increase the expression of iNOS and COX-2 (Fig. 1G). Similarly, in endothelial cells iNOS and COX-2 protein expression was not increased by hypoxia and TNF-α (Fig. 1H).

Since iNOS and COX-2 is co-induced in adipocytes in the presence of hypoxia and TNF-α, an immunoprecipitation experiment was performed to determine whether

iNOS physically interacts with COX-2 (Supplementary Figure 1). The data suggest that in endogenous condition, iNOS does not bind to COX-2 in adipocytes.

**TNF-α alters the gene expression of cytokines in normoxia and hypoxia**

In 3T3-L1 adipocytes, the gene expression of pro-inflammatory genes such as interleukin 6 (IL 6), serum amyloid A1 (SAA1), regulated on activation, normal T cell expressed and secreted (Rantes) and monocyte chemoattractant protein1 (MCP1) was similarly increased after stimulation with TNF-α in normoxic and hypoxic conditions (Fig. 2). In contrast, the gene expression of anti-inflammatory adiponectin and leptin was decreased in the presence of TNF-α in normoxia and to a same extent by hypoxia. Hypoxia alone increased the gene expression of plasminogen activator inhibitor type 1(PAI-1) by 3-fold and the expression levels were similar when cells were treated with TNF-α in hypoxia (Supplementary Figure 2A). The gene expression of vascular endothelial growth factor (VEGF), a pro-angiogenic molecule, was increased only by hypoxia and TNF-α had no effect on its expression (Supplementary Figure 2B).

**iNOS and COX-2 inhibitors reduce nitrite levels**

Since iNOS was significantly increased in adipocytes by TNF-α during hypoxia, the nitrite levels were measured in 3T3-L1 adipocytes under these conditions. As compared with only hypoxia, the nitrite levels were not significantly increased in presence of hypoxia with TNF-α (Fig. 3A). The increased nitrite levels in hypoxia were significantly reduced by iNOS specific inhibitor 1400W (Fig. 3A). Interestingly, the COX-2 specific inhibitor NS398 also reduced the nitrite level (Fig. 3B).

**TNF-α and hypoxia increases $PGE_2$ levels**

The expression of COX-2 was significantly upregulated by TNF-α during hypoxia, hence the functional activity of COX-2 was evaluated and its enzymatic product $PGE_2$ was measured in similar conditions. Treatment of adipocytes with TNF-α in the presence of hypoxia significantly increased the levels of $PGE_2$. In the presence of iNOS specific inhibitor, 1400W, the $PGE_2$ levels were not significantly reduced (Fig. 3C). In contrast, the selective COX-2 inhibitor, NS398, significantly reduced the increased levels of $PGE_2$ in hypoxia (Fig. 3D).

**TNF-α and hypoxia decreases PGC-1α mRNA**

We then investigated the gene expression of some pro-inflammatory transcription factors and its co-activators. ATF3, a pro-inflammatory transcription factor was increased by TNF-α in the presence of normoxia (Fig. 4A). Hypoxia alone or together with TNF-α increased the gene expression of ATF3 and the expression levels were similar. The gene expression of PPARγ, a transcription factor required for adipocyte homeostasis, was reduced by 2-fold upon TNF-α treatment in the presence of normoxia and was further reduced, albeit non-significantly, with hypoxia (Fig. 4B). Nrf-1, a transcription factor involved in mitochondrial biogenesis, was reduced by 1.6-fold with TNF-α in the presence of normoxia and to the same extent by hypoxia (Fig. 4C). The gene expression of PGC-1α, a co-activator of transcription factor which is also involved in mitochondrial biogenesis, was reduced by 4-fold after treatment with TNF-α in the presence of normoxia (Fig. 4D). Hypoxic condition reduced the gene expression of PGC-1α to the same extent. Interestingly, treatment of adipocytes with TNF-α during hypoxia further decreased the expression of PGC-1α (by 3-fold) in comparison with normoxia and TNF-α.

## Discussion

Development of adverse pathophysiological conditions in obesity involves inflammation [3,22]. Heightened levels of TNF-α in obese individuals triggers adipocyte dysfunction [23] and mediates insulin resistance [4,22,24]. Macrophages within the adipose tissue are supposedly the source of TNF-α [2]. In this study, we mimicked the pro-inflammatory environment in adipose tissue by stimulating adipocytes under normoxia and hypoxia with exogenous TNF-α. In both normoxic and hypoxic conditions, TNF-α increased the expression of several pro-inflammatory and acute-phase molecules such as IL-6, SAA, Rantes, MCP-1, PAI-1 and reduced the expression of cytokines such as adiponectin and leptin. Furthermore, TNF-α stimulated the expression of iNOS and COX-2 in normoxia and interestingly, hypoxia potentiated the effect of TNF-α in inducing iNOS and COX-2 expression in both white and brown adipocytes.

It is well established that hypoxia in obese adipose tissue triggers inflammation and pockets of hypoxia colocalize with the presence of macrophages [11,15,25]. The robust increase in the expression of iNOS and COX-2 after TNF-α stimulation during hypoxia was observed specifically in adipocytes (white and brown) and not in preadipocytes and endothelial cells. Cardiomyocytes treated with IL-1β in the presence of hypoxia reduced the expression of iNOS and displayed reduced levels of nitrite [26]. This suggests that inflammatory stimuli and hypoxia driven induction of iNOS and COX-2 is evident in adipocytes and is clearly cell type dependent. A crosstalk between iNOS and COX-2 has been reported and a physical interaction between the molecules was shown using an overexpression system in HEK293T cells [20]. However, in our work, we could not detect a physical interaction under endogenous setting even with high expression levels of iNOS and COX-2. Murine macrophages stimulated with IFN-γ increased the expression of iNOS mRNA

however nitrite did not accumulate in culture supernatant [27]. In our experiments, hypoxia caused a significant rise in nitrite level even with low iNOS expression. However, hypoxia plus TNF-α did not significantly augment the nitrite levels further even with elevated iNOS expression levels. Since oxygenation is critical for nitric oxide formation and it has been suggested that oxygenation mediates the accumulation of nitrite in culture supernatant [27]. It is thus possible that the conserved release of nitrite in presence of hypoxia and TNF-α was due to the absence of re-oxygenation in our experimental setting. The inhibitor studies using iNOS selective inhibitor, 1400W, indicate that the iNOS was involved in nitric oxide release in hypoxia. Surprisingly, the levels of nitrite were lowered when COX-2 specific inhibitor NS398 was used. The COX-2 product prostaglandin $E_2$ was only lowered by NS398 and not by 1400W. These findings suggest that in differentiated adipocytes COX-2 may be upstream of iNOS in the signalling cascade.

Decreased mitochondrial biogenesis increases iNOS expression in adipocytes [28] and PGC-1α, a transcriptional coactivator, is important for mitochondrial biogenesis [29]. Chronic exposure of adipocytes to TNF-α under normoxia causes insulin resistance and decreases PGC-1α expression [24]. In our study, the expression of PGC-1α was significantly lowered after adipocytes were exposed to hypoxia in the presence of TNF-α. In white adipose tissue, PGC-1α stimulates its own transcription by coactivating the transcription factor PPARγ [30]. In our study, the expression of PPARγ was also decreased by hypoxia in the presence of TNF-α. Thus, the lower expression of PPARγ could be the linked to the low PGC-1α expression. It has been shown that mice with iNOS deletion in brown adipose tissue have an enhanced expression of PGC-1α [31]. This can be extrapolated to our finding and suggest a probable link between the expression of iNOS and PGC-1α. Moreover, leptin increases PGC-1α expression [32]. In our study, expression of leptin was decreased

after adipocytes were exposed to hypoxia with TNF-α. In adipocytes, the mitochondrial function is essential for adiponectin synthesis [33]. The low expression of adiponectin in our study indicates the presence of mitochondrial dysfunction by hypoxia and TNF-α. Thus, the increased expression of iNOS and COX-2 along with decreased expression of adiponectin, leptin, PPARγ and PGC-1α contribute to adipocyte dysfunction probably via altering the mitochondrial function.

At a pathophysiological level the in vitro findings in this work suggest that hypoxia within adipose depots in obese patients could further worsen an existent low-level inflammation by amplifying the inflammatory response. Therefore, methods to reduce hypoxia within obese adipose tissue may be of importance. In this regard, improving vascularization and thus oxygenation using anti-hypertensive drugs such as angiotensin-converting enzyme inhibitors which normalize rarefied microvessel density [34] could be an useful approach. Moreover, practicing healthy lifestyle can promote oxygenation and reduce the tissue inflammatory load. Thus, the reduction in hypoxia-mediated inflammation and lowering of iNOS and COX-2 expression in adipose tissue could prevent or even reverse the worsening of obesity-associated pathophysiological conditions.

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of Zurich, University Hospital of Zurich, Hartmann Müller Stiftung, and the Swiss National Science Foundation.

**Figure Legends**

**Fig. 1: Gene and protein Expression of iNos and Cox-2.** (A) Differentiation of 3T3-L1 preadipocytes to adipocytes. (B) 3T3-L1 preadipocytes, (C) 3T3-L1 adipocytes and (D) brown adipocytes were stimulated without or with TNF-α for 24 h in the presence of normoxia (21% $O_2$) or hypoxia (1% $O_2$). Gene expression of iNos and Cox-2 were examined using real time qPCR. *$p<0.05$ vs. normoxia and TNF-α. N=3-5. (E) Cell lysates from 3T3-L1 adipocytes, (F) brown adipocytes, (G) 3T3-L1 preadipocytes and (H) mouse aortic endothelial cells were immunoblotted with specific iNOS or COX-2 antibodies. The protein loading was checked using Erk2 antibody. N=3.

**Fig. 2: Gene expression of cytokines.** 3T3-L1 adipocytes were stimulated without or with TNF-α for 24 h in the presence of normoxia (21% $O_2$) or hypoxia (1% $O_2$). Gene expression was examined using real time qPCR. N=3-4.

**Fig. 3: Measurement of nitrite and prostaglandin $E_2$.** The levels of nitrite were measured in the conditioned media using Griess reagent. (A) The 3T3-L1 adipocytes in hypoxia were pre-treated either with 1400W or (B) with NS398 in the presence or absence of TNF-α for 24 h. *$p<0.05$ vs. hypoxia. N=3.

The levels of prostaglandin $E_2$ ($PGE_2$) were measured from the conditioned media. (C) The 3T3-L1 adipocytes in hypoxia were pre-treated either with 1400W or with (D) NS398 in the presence or absence of TNF-α for 24 h. *$p<0.05$ vs. hypoxia. N=3.

**Fig. 4: Gene expression of transcription factors or its co-activators.** 3T3-L1 adipocytes were stimulated without or with TNF-α for 24 h in the presence of normoxia (21% $O_2$) or hypoxia (1% $O_2$). (A) Gene expression of ATF3, (B) PPARγ,

(C) Nrf-1 and (D) PGC-1α were examined using real time qPCR. *p<0.05 vs. normoxia with TNF-α. N=4-6.

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Figure

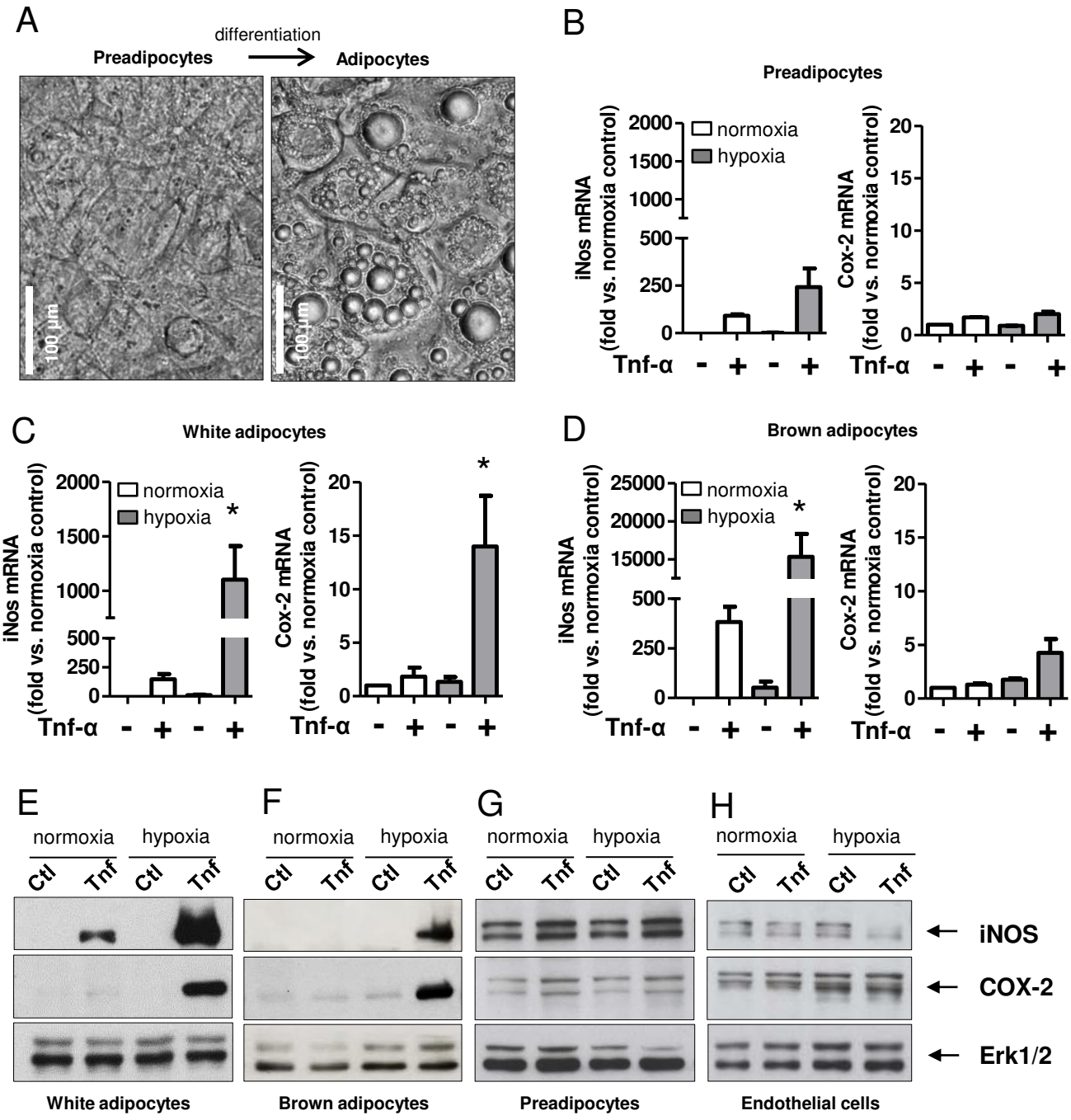
A
differentiation
Preadipocytes
Adipocytes
100 µm
100 µm
B
Preadipocytes
iNos mRNA (fold vs. normoxia control)
normoxia
hypoxia
Cox-2 mRNA (fold vs. normoxia control)
Tnf-α - + - +
C
White adipocytes
*
D
Brown adipocytes
E
F
G
H
normoxia
hypoxia
Ctl
Tnf
iNOS
COX-2
Erk1/2
White adipocytes
Brown adipocytes
Preadipocytes
Endothelial cells

Fig. 1

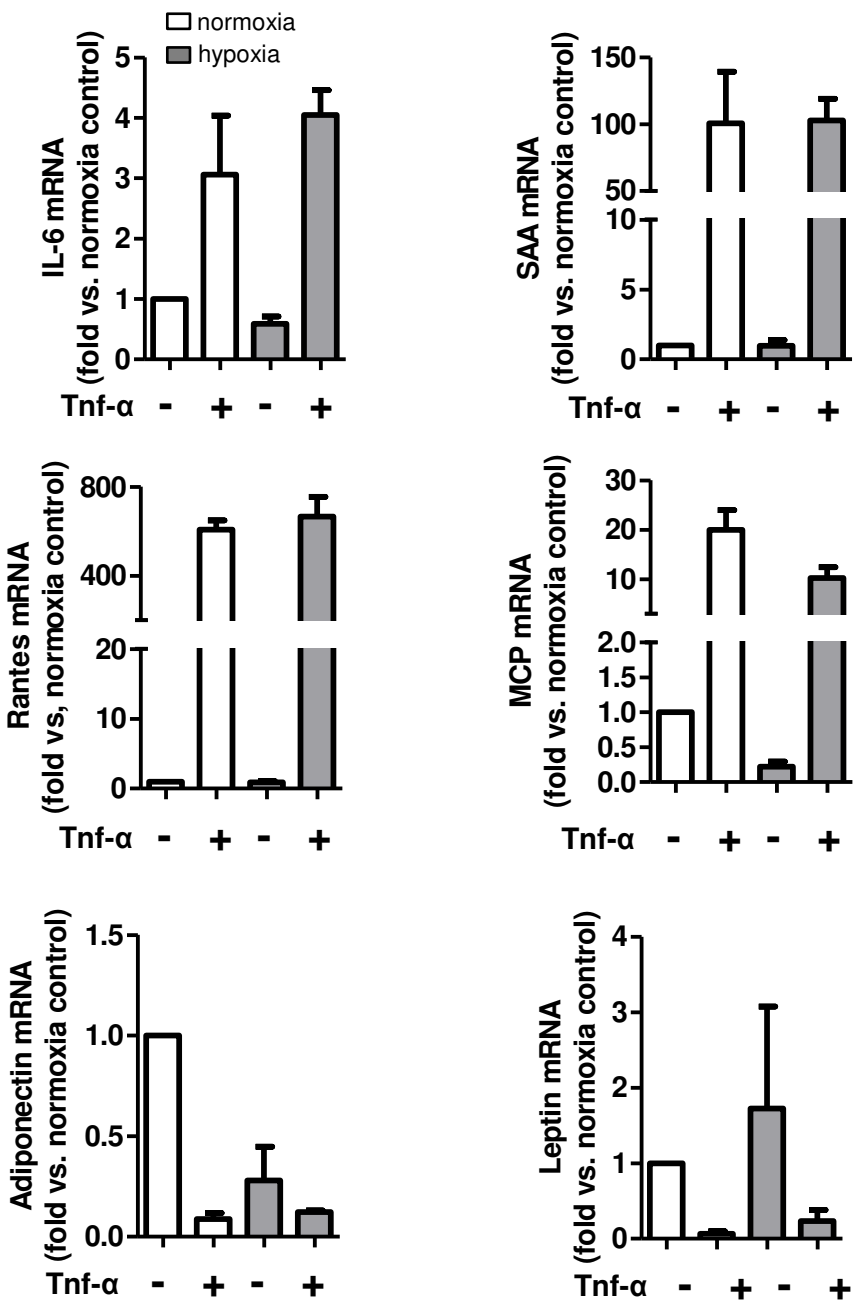
normoxia
hypoxia
IL-6 mRNA (fold vs. normoxia control)
SAA mRNA (fold vs. normoxia control)
Rantes mRNA (fold vs, normoxia control)
MCP mRNA (fold vs. normoxia control)
Adiponectin mRNA (fold vs. normoxia control)
Leptin mRNA (fold vs. normoxia control)
Tnf-α - + - +

Fig. 2

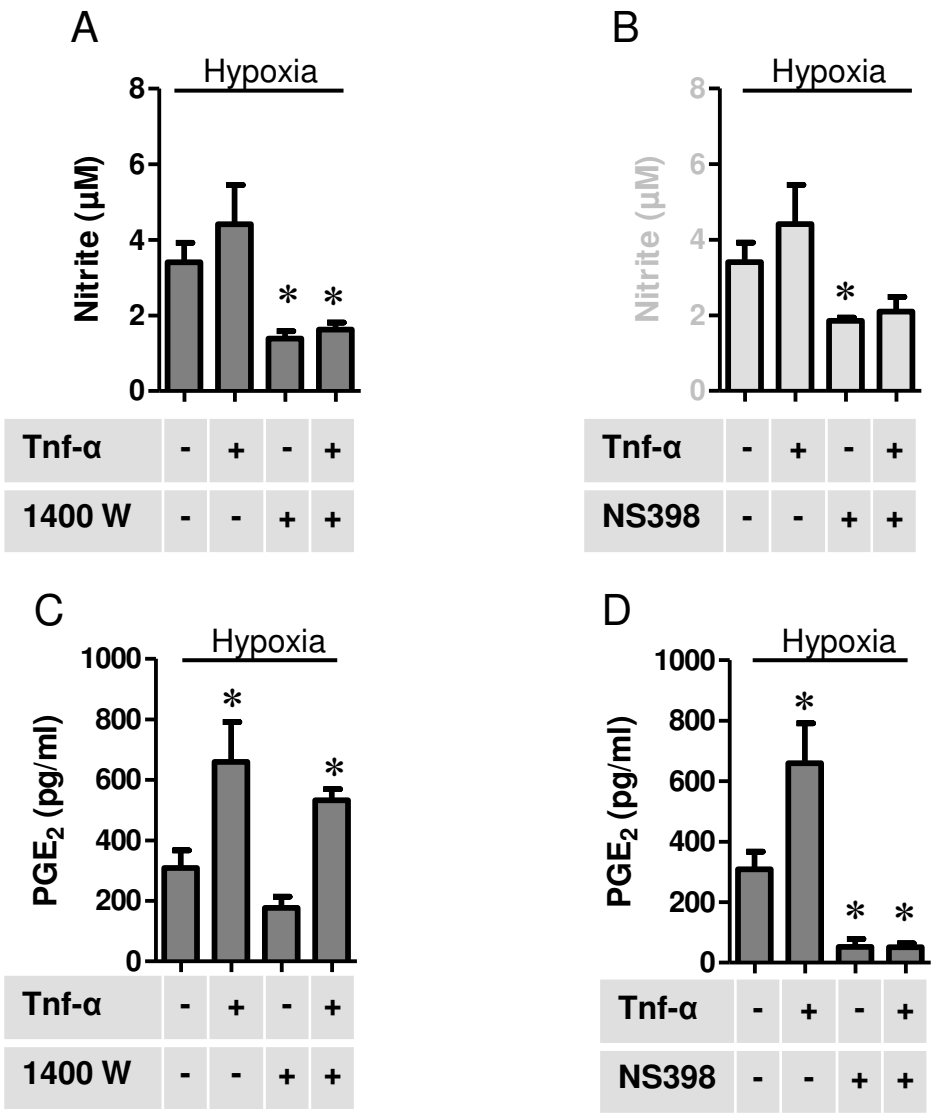
A
Hypoxia
Nitrite (µM)
8
6
4
2
0
*
*
Tnf-α - + - +
1400 W - - + +
B
Hypoxia
Nitrite (µM)
8
6
4
2
0
*
Tnf-α - + - +
NS398 - - + +
C
Hypoxia
PGE2 (pg/ml)
1000
800
600
400
200
0
*
*
Tnf-α - + - +
1400 W - - + +
D
Hypoxia
PGE2 (pg/ml)
1000
800
600
400
200
0
*
*
*
Tnf-α - + - +
NS398 - - + +

Fig. 3

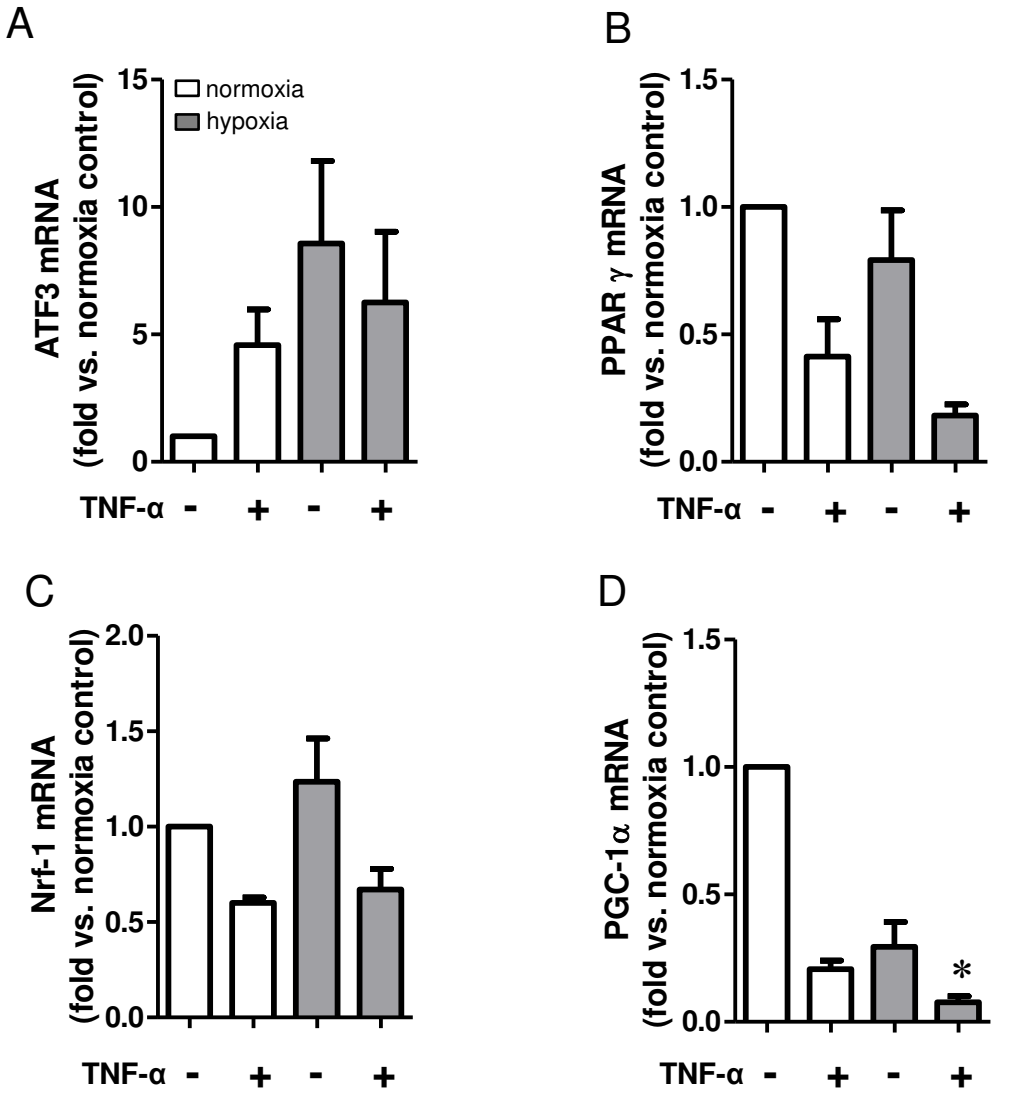
A
normoxia
hypoxia
ATF3 mRNA
(fold vs. normoxia control)
15
10
5
0
TNF-α - + - +
B
PPAR γ mRNA
(fold vs. normoxia control)
1.5
1.0
0.5
0.0
TNF-α - + - +
C
Nrf-1 mRNA
(fold vs. normoxia control)
2.0
1.5
1.0
0.5
0.0
TNF-α - + - +
D
PGC-1α mRNA
(fold vs. normoxia control)
1.5
1.0
0.5
0.0
*
TNF-α - + - +

Fig. 4

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